

REMARKS

After amendment, claims 1-40 and 45-50 remain pending in the present application. Claims 41-44 were previously cancelled. Claims 47-50 are new. The claims previously have been amended to place the application in condition for allowance based upon the unexpected activity of DOT in inhibiting drug resistant forms of HIV. Support for the amendments to the claims can be found throughout the original specification, including the examples and originally filed claims. No new matter has been added by way of the present amendment.

The Examiner has withdrawn a number of objections and rejections of the instant application. The Examiner has also rejected the previously pending claims for the reasons which are set forth in the August 2007 office action on pages 3-10. Applicants shall address each of the Examiner's concerns in the sections which are presented hereinbelow. It is respectfully submitted that the application after amendment, is in condition for allowance.

The 35 U.S.C. §102 Rejection

The Examiner has maintained the rejection of claims 23-29 and 47 under 35 U.S.C. §102 over Belleau, et al., U.S. patent no. 7,119,202 ("Belleau"), for the reasons which are stated in the office action on pages 3-4. Applicants respectfully traverse the Examiner's rejection.

The claims of the present invention are directed to the use of DOT or its prodrug for the treatment of a drug-resistant form of HIV in a patient. Belleau does not disclose the treatment of a drug-resistant form of HIV utilizing DOT or its prodrug analog. It is noted that Belleau does not even mention drug-resistant forms of HIV and certainly do not disclose DOT or its prodrug analogs for treatment of same. An electronic search of the Belleau patent documents show that the specification does not disclose or suggest the

use of the compounds disclosed therein with drug-resistant forms of HIV. Such a disclosure is nowhere to be found in the cited patent documents.

Moreover, Belleau provides absolutely no biological studies or activity from which one of ordinary skill could glean that DOT was an agent which could be used effectively to treat HIV strains which are resistant to 3TC and/or AZT either alone or in combination with an additional anti-HIV compound as claimed.

Belleau does not provide any biological data whatsoever and doesn't even mention drug resistant forms of HIV. Without any additional disclosure, it is respectfully submitted that the Examiner has not made out a cogent case that the presently claimed invention is anticipated by Belleau. Noted also is the rather detailed biological data in the present specification (see tables 1-5 on pages 17-20) which clearly evidence that compounds (DOT or prodrug forms) which are disclosed herein exhibit activity against drug resistant forms of HIV, including multiple drug resistant forms and that the compounds which are disclosed and claimed herein represent a viable therapeutic approach, alone or preferably in combination with another anti-HIV agent which exhibits inhibition of HIV by a mechanism other than that of DOT. As further evidence of the distinction of the present application as against the prior art, Applicants respectfully enclose a copy of the reference *Antimicrob. Agents Chemother.*, June 2007, pp. 2078-2084 of which the co-inventors of the present inventors are co-authors. The enclosed reference unequivocally evidences the unexpected activity which DOT exhibits *vis-à-vis* drug resistant forms of HIV, and in particular, reverse transcriptase. Indeed, this reference clearly evidences that DOT exhibits significantly greater/more potent activity against viruses with a number of mutations to reverse transcriptase in HIV. In essence, the present invention represents a clear advance in the art of treating HIV infections. This was neither disclosed or even obliquely mentioned by Belleau.

For the above reasons, it is respectfully submitted that the claims of the present application are not anticipated by Belleau.

The 35 U.S.C. §103 Rejection

The Examiner has rejected the previously filed claims under §103 as set forth in the office action on pages 4-6 as being obvious/unpatentable over Liotta, Belleau or Liotta and the Merck Manual of Diagnosis and Therapy, 17th edition (“Merck”) for the reasons which are set forth therein. For the reasons which are presented below, Applicants respectfully submit that the presently pending claims are non-obvious and patentable over the cited art.

The present invention is directed to methods of or compositions for use in treating drug resistant (primarily 3TC and/or AZT drug resistant) forms of HIV (based upon mutations to reverse transcriptase in HIV) using the claimed compounds (DOT or a prodrug form of DOT) alone or preferably in combination with another anti-HIV agent. Thus, the present invention is directed to the unexpected activity of DOT or its analogs in exhibiting significant inhibition of various drug resistant strains of HIV. The aforementioned biological activity is presented in the specification of the present application in tables 1-5 on pages 17-20. The Examiner rejected the previously pending claims as being obvious over the cited art. Applicants respectfully submit that the instant claims are non-obvious over that art.

It is noted here that the references upon which the Examiner has relied for making his obviousness rejection provide insufficient teachings for providing a cogent argument that the present invention is obvious and consequently, unpatentable. Moreover, those references, Liotta and Belleau were filed 17 (seventeen) and 19 (nineteen) years ago respectively. To date, despite the Examiner’s reliance on these references as teaching the present invention, there is no evidence that the basic thymine analog which is claimed herein has been utilized as an effective analog for the treatment of HIV. The present invention is non-obvious over the disclosed teachings. Moreover, the only reference which details the activity of DOT in treating drug resistant forms of HIV is the attached/enclosed reference, *Antimicrob. Agents Chemother.*, June 2007, pp. 2078-2084, which Applicants cite in support of the patentability of the instant application.

In the first instance, the Examiner has rejected claims 2-4, 13-16, 31-34 and 45-47 as being unpatentable over the disclosure of Liotta. It is the Examiner's view that because Liotta teaches treating HIV infections generally, it would be obvious to use DOT or its analogs to treat drug-resistant forms of HIV. Applicants respectfully traverse the Examiner's rejection.

Prior to the disclosure in the present application, the art did not recognize, nor could one of ordinary skill predict that DOT or a related analog as claimed would be particularly effective in treating HIV which was resistant primarily to 3TC and/or AZT. That was simply unknown and unknowable unless someone took the time to test DOT against a number of drug resistant strains of HIV. Not only did Liotta *not* test the disclosed compounds against drug resistant strains of HIV, Liotta does not even mention drug resistant forms in the specification. Given the absence of disclosure to that effect in Liotta, it simply cannot be said with any measure of conviction that the present claims are obvious over Liotta. Liotta only provides evidence of the *somewhat* marginal *in vitro* activity of DOT against HIV (See Table 1, column 17 of Liotta). Without adequate testing, one cannot simply draw any conclusion with respect to the activity of a drug against a drug-resistant viral strain. It is simply untenable to suggest that Liotta somehow renders the present invention obvious.

It is noted that the *in vitro* activity against HIV of compounds which are used in the present invention is shown to be somewhat marginal in Liotta (10-20 fold lower activity than other compounds tested). This limited activity against HIV may explain why, a number of years after the basic application for Liotta was filed (in 1991), and until the filing of the present application, DOT was not viewed as a clinically relevant compound for the treatment of HIV. It was simply not used in clinic and never became a clinically relevant drug. Because of the work by the present inventors, DOT is now viewed as a potentially viable treatment for drug resistant forms of HIV and clinical work is ongoing to provide pharmacokinetic data and additional clinical data to provide DOT

as clinically relevant therapeutic agent. The invention of the present application is clearly non-obvious over the teachings of Liotta.

Turning to the rejection of claims 2-4, 13-16, 31-34 and 45-47 as being obvious over Belleau, it is respectfully submitted that the disclosure of Belleau does not in any way render the present invention obvious for the same reasons Liotta does not render the present invention obvious. Belleau is directed to a number of nucleoside analogs, a number of which are posited as being active against HIV. Belleau provides absolutely no biological activity against HIV using DOT or a prodrug form. Belleau does not suggest nor even mention drug resistant forms of HIV or that DOT may be a useful agent against same. Belleau provides absolutely no motivation to provide the present invention. Without providing any biological activity against HIV and without suggesting the use of DOT or a related analog as claimed against a drug resistant form of HIV or even mentioning a drug resistant form of HIV, it is respectfully submitted that one of ordinary skill in the art could not possibly have recognized the present invention from the disclosure of Belleau.

Further noted herein is the fact that Belleau provides no evidence of efficacy of DOT, and, as in the case of Liotta, no attempt has been made to establish the clinical relevance of DOT by the inventors of Belleau or Liotta. Indeed, the activities of the present inventors are the first concerted effort to establish the clinical relevance of DOT as a treatment for drug resistant forms of HIV, an important therapeutic subclass. The present invention is clearly patentable over Belleau.

We now turn to the Examiner's rejection of claims 5-7, 17, 18, 23-29, 35 and 36 as being patentable over Liotta, in view of Merck. Applicants have reviewed the disclosure of Liotta in view of Merck and the Examiner's discussion on pages 9-11 of the August 2007 office action and conclude that the present invention is patentable over the combined disclosures of those cited references.

For the reasons which have been detailed hereinabove, it is respectfully submitted that Liotta fails to suggest the present invention. In fact, Liotta does not even mention drug resistant forms of HIV, let alone their treatment with the claimed compounds. The disclosure of DOT and its putatively somewhat marginal *in vitro* activity as tested, would teach the routineer away from using DOT as an anti-HIV compound. No further development would ensue from a reading of Liotta. Indeed, this is precisely what happened, inasmuch as the teachings of Liotta gave rise to complete inactivity by the inventors in establishing the therapeutic relevance of DOT against HIV. Prior to the present invention, as taught by Liotta, DOT was not a relevant viable clinical drug for treating HIV. With the present invention, DOT now is clinically relevant.

Turning to Merck, this reference, generic in nature, does not in any way obviate the deficient disclosure of Liotta inasmuch as Merck also fails to suggest that the nucleoside compounds of the present invention may be used to treat drug resistant forms of HIV and in particular, 3TC and/or AZT resistant strains of HIV. Without so much as an oblique mention of drug resistant forms of HIV, the Examiner has concluded that Liotta in view of Merck can be combined to teach combination therapy using DOT or a related compound to treat specific drug resistant forms of HIV. Without so much as even an oblique reference to same, it is respectfully submitted that the present invention is non-obvious over the combined teachings of Liotta and Merck, especially given the *years of inactivity* by the inventors of Liotta and Belleau in failing to establish the clinical relevance of DOT in treating HIV infections, precisely because they believed DOT to be insufficiently active as a compound having clinical potential.

Thus, where, as *here*, the prior art is absolutely silent on both the teaching and/or the suggestion of the claimed invention, patentability is instilled. This is especially true where the conduct of the inventors is such that there is a complete failure *for years* to establish an interpretation of the teachings upon which Examiner relies in making the art rejection. There is simply no disclosure or suggestion in any of the prior art references, alone or in combination to use DOT and related analogues as claimed in the treatment of drug resistant forms of HIV, because those teachings were unavailable prior to the

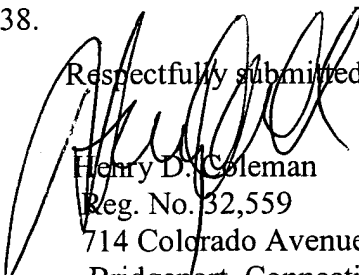
present invention. It is respectfully submitted that the instant claims are now fully compliance with the requirements of 35 U.S.C.

For all of the above reasons, it is respectfully submitted that the claims are patentable. Consequently, it is respectfully submitted that the pending claims are in condition for allowance and such action is earnestly solicited.

No fee is due for the presentation of the present amendment/response. Three dependent claims were added. Four dependent claims were previously cancelled from the present application. Please charge any additional fee due or credit any overpayment previously made to Deposit Account No. 04-0838.

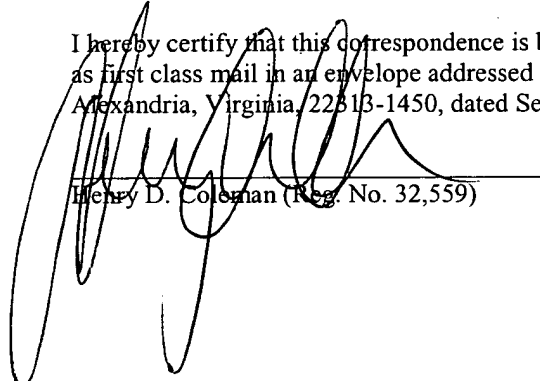
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Biochemical Studies on the Mechanism of Human Immunodeficiency Virus Type 1 Reverse Transcriptase Resistance to 1-(β -D-Dioxolane)Thymine Triphosphate[†]

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A large panel of drug-resistant mutants of human immunodeficiency virus type 1 reverse transcriptase (RT) was used to study the mechanisms of resistance to 1-(β -D-dioxolane)thymine triphosphate (DOT-TP) and other nucleotide analogs. RT containing thymidine analog-associated mutations (TAM) or RT with a T69S-SG insertion in combination with TAM removed 3'-azido-3'-deoxythymidine-5'-monophosphate or tenofovir more efficiently than DOT-monophosphate from chain-terminated DNA primer/template through ATP-mediated pyrophosphorolysis. For non-ATP-dependent discrimination toward DOT-TP, high levels of resistance were found for RT bearing the Q151M mutation with family mutations, while RT bearing only the M184V or the Y115F mutation conferred no resistance to DOT-TP. A lower degree of resistance to DOT-TP than to tenofovir diphosphate or carbovir-TP was found for RT containing the K65R mutation. In the present studies, 1-(β -D-dioxolane)guanine triphosphate, another nucleotide with a dioxolane sugar moiety, showed a resistance profile similar to that of DOT-TP. The results suggest that DOT, compared with other approved nucleoside analogs, is overall more resilient to mutations such as TAM, M184V, and K65R, which are commonly found in viruses derived from subjects failing multinucleoside therapy.

Nucleoside analog reverse transcriptase inhibitors (NRTI) remain the central class of drugs included in highly active antiretroviral therapy (HAART) cocktails used in the treatment of human immunodeficiency virus type 1 (HIV-1) infections. NRTI in their metabolically activated form, NRTI-5'-triphosphate (NRTI-TP), inhibit HIV-1 reverse transcriptase (RT) viral synthesis by competing with the natural 2'-deoxy nucleosides-5'-triphosphates (dNTP) and/or by chain termination, since they lack the 3'-hydroxyl group (11). Drug resistance is the major limitation of NRTI treatment, as has been reported for all classes of HIV-1 inhibitors (16, 17, 31). Our present understanding is that NRTI resistance occurs by mechanisms requiring that mutant RTs discriminate against the NRTI better than wild-type RTs, either before or after the incorporation of NRTI into the primer strand. The latter mechanism, referred to as pyrophosphorolysis, excision, or primer unblocking, involves an enhanced removal of the incorporated NRTI-monophosphate (MP), e.g., 3'-azido-3'-deoxythymidine-5'-MP (AZT-MP), thus, freeing the primer strand for further elongation (1, 24, 25). ATP at a physiological concentration of 3.2 mM (36) is considered the major pyrophosphate donor in cells for this mechanism (20, 24). The mutations in HIV-1 RT generally associated with this mechanism are M41L, D67N, T70R, L210W, T215Y/F, and/or K219Q, where T215Y is the key mutation. These mutations were first found in individuals treated with AZT or 2',3'-dideoxy-2',3'-

didehydrothymidine (d4T) and are termed thymidine analog-associated mutations (TAM). The hypothesis is that the side chain switch of residue 215 threonine to tyrosine or phenylalanine improves the position of the ATP molecule for the catalytic attack of the incorporated NRTI-MP. Thus, the NRTI-MP is more easily removed as a dinucleoside tetraphosphate product (5, 7, 23, 29). Enhanced primer unblocking of NRTI-MP can also be achieved with mutations at residue 69 in the HIV polymerase as a two-amino-acid insertion (19, 20, 23). In contrast to the primer unblocking mechanism, mutations such as M184V, K65R, and Q151M are involved in direct substrate discrimination before the incorporation of NRTI (18, 31, 33). In fact, some of these mutations have been reported to suppress primer unblocking (31).

In the decade since HAART was introduced, a wealth of data has accumulated by which trends in the prevalence of drug resistance mutations can be established. Virus samples from treated individuals experiencing virologic failure have demonstrated the following prevalence of NRTI mutations: M184V, 48%; T215Y/F (representing TAM), 45%; T69D, 6.4%; L74V, 6%; Q151M, 2.7%; K65R, 1.9%; Y115F, 1.1%; and T69S-XX insertions, 0.8% (Stanford HIV Drug Resistance Database, <http://hivdb.stanford.edu>). However, since protocols have been updated from previous regimens consisting of AZT/d4T with β -L-2',3'-dideoxy-3'-thiacytidine or 2',3'-dideoxyinosine to the more up-to-date regimens with tenofovir (TFV)-emtricitabine (Emtriva [FTC]) or β -L-2',3'-dideoxy-3'-thiacytidine and abacavir, there has been a slight decrease in the frequency of mutations TAM, T69D, Q151M, and residue 69 insertions, while there is a minor trend toward higher frequencies of mutations M184V, K65R, and Y115F (R. W. Shafer, personal communications).

Hence, there is still a need for additional NRTI that, besides

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being potent and less toxic, have a greater genetic barrier to the current common resistance mutations. Moreover, such an NRTI should be suitable for the inclusion of future combination therapies. Acknowledging the need to replace the thymidine kinase (TK)-dependent AZT and d4T therapies, which can be toxic on prolonged use and not active against the frequent TAM, this report focuses on (–)-(2*R*,4*R*)-1-(2-hydroxy-methyl-1,3-dioxolan-4-yl)thymine (also known as DOT), a potent and thymidine kinase-1 dependent NRTI (8, 30). The structure of DOT differs from that of other NRTI since it contains a dioxolane instead of a sugar moiety (8). Another dioxolane nucleoside, β-*D*-dioxolane-guanine (DXG), the deaminated form of the prodrug β-*D*-2,6-diaminopurine dioxolane (DAPD), has selective anti-HIV activity and is nontoxic to mammalian and human cells (12, 35). DXG was found in cell culture studies to be active against HIV-1 strains containing TAM and/or M184V mutations in the Pol gene product (15). These DXG resistance results support data obtained with DOT, using structure modeling (9).

The focus of this work was to probe the biochemical mechanisms of resistance to DOT-triphosphate (TP) using a novel nonradioactive polymerase assay. A panel of purified HIV-1 RT containing mutations (TAM and M184V) that have been commonly observed in clinical samples obtained from HIV-infected individuals treated with nucleoside analogs was used. In addition, RT with mutations that are involved in multidrug resistance (Q151M and T69S-SG insertion) or that are increasing in prevalence, although still at a low frequency (K65R and Y115F), were also studied. The overall objective was to elucidate the level of resistance to DOT-TP compared to those of other approved NRTI-TP and DXG-TP for a broad range of RT mutants and to study the mechanism of resistance to these analogs.

(This work was presented in part at the 13th Conference on Retroviruses and Opportunistic Infections, Denver, CO, 5 to 8 February 2006.)

MATERIALS AND METHODS

Site-directed mutagenesis and expression and purification of HIV-1 RT. Site-directed mutagenesis of the RT from different HIV-1 wild-type backgrounds (Table 1) were generated using a QuikChange method (Stratagene), but different sources of expression vectors and purification methods were used. The first eight RT described in Table 1 were prepared using the isolate HXB2(a) background based on the pKK233-2 expression vector (kindly provided by D. K. Stammers) and were expressed and purified to heterodimer p66/p51 as previously described, using anion and cation exchange chromatography (20, 34). A C-terminal histidine-tagged expression vector similar to p6HRT-PROT based on the expression vector pT5m (kindly provided by S. H. Hughes) was used to express the HXB2(b) and 41L/67N/70R/215Y/219Q mutant RTs. The plasmid simultaneously expressed HIV-1 RT p66 and HIV-1 protease and thereby expresses both the RT p66 and p51 forms, as p66 is processed by the protease. A HiTrap chelating Ni²⁺ column (Amersham/GE Health Care) purification method was used (3). The same expression vector and purification method were also used to clone and purify the pNL4-3 wild-type RT and the Y115F mutant. The K65R RT and its equivalent wild-type isolate LAI RT were supplied as purified homodimer p66 form RT (a generous gift from P. Meyer, W. A. Scott, and J. W. Mellors). The purification of these N-terminal histidine-tagged products has previously been described (24). All of the RT products had a purity of >90% according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. All the preparations, except for the K65R mutant and the LAI wild-type RTs, were in p66/51 heterodimeric form, with the p66 form at levels of 55 to 75% and the p51 at levels of 25 to 45%.

Heteropolymeric DNA polymerase assay. Two different nonradioactive RT assays were used, the DNA polymerase assay and the poly(rA)/oligo(dT) (prA/

odT) assay, to study the NRTI-TP inhibition of the purified RT mutants. Separate kit components such as covalently linked DNA microtiter plates or prA plates (from a high-sensitivity type RT assay) and tracer solution (alkaline phosphate [AP]-conjugated anti-bromodeoxyuridine [BrdU] antibody) were obtained from Cavid Tech, Uppsala, Sweden.

The DNA polymerase assay is based on a DNA primer with a specific sequence that is covalently bound to the well of the 96-well microtiter plate; an 18-base 5'-GTC-CCT-GTT-CCG-GCG-CCA-3' sequence linked at the 5' end to the primary amine by a C6 spacer arm. The amount of primer bound was approximately 10 ng/well (110 nM). The RT assay reaction mixture (total volume 150 μl/well) contained HEPES 50 mM (pH 7.3); MgCl₂, 10 mM; Triton X-100, 0.5%; bovine serum albumin, 0.1 mg/ml; dATP, dGTP, dCTP, and 5-bromo-2'-deoxyuridine-5'-triphosphate (BrdUTP), 1.0 μM each (where BrdUTP replaces TTP) (all from Sigma); and a 50-base DNA template (5'-AAA-AAA-AAA-AAA-GTC-AGT-CAG-TCA-GTC-AGT-CAT-GGC-GCC-CGA-ACA-GGG-AC-3'), 50 ng/well (190 nM) (i.e., a 5'-A₁₂-3' tail with a 5'-(GTCA)₃-3' repeat as template) (Integrated DNA Technologies). The reason for positioning the pA₁₂ region at the template 5' end was to have a variable sequence before the major signal for product detection with tracer antibody. To obtain a significant ATP primer unblocking reaction in the assay, the ATP (Amersham/GE Health Care) concentration was set to 5 mM. We found the ATP effect for the 41L/67N/70R/210W/215Y mutant resistance to AZT-TP and TFV-diphosphate (DP) to vary in a linear fashion between 0 and 6 mM and to plateau with maximum ATP-dependent resistance at approximately 8 mM ATP (data not shown).

The reaction was started with the addition of RT in the 30-to-100-pM range. The recombinant HIV-1 polymerases had an activity within 1,500 to 3,000 U/mg as defined by the standard Lenti RT assay (Cavid Tech) (10). To achieve reproducible kinetics between wild-type and mutant RTs, approximately 1.5 to 4.5 mU/well of each enzyme was used. It should be noted that a 10-fold broader range had very limited influence on the 50% inhibitory concentration (IC₅₀) values for the NRTI-TP (data not shown). In order to further improve the comparison between wild-type and mutant RTs, the same sources of vectors, purification methods, and p66/p51 dimerization were used as described in Table 1. The RT reaction mixture was incubated at 33°C for 180 min and terminated by a water wash of the plates. Since the anti-BrdU antibodies (tracer AP-conjugated) bound more favorably to incorporated BrdUMP as a single-strand rather than a double-strand DNA product, incubation with 2 M NaOH (3 times 200 μl/well for 5 min) was performed to dehybridize the DNA template. After a thorough wash with water, tracer was added and incubated for 90 min at 33°C. The detection step for color absorbance at 405 nm was performed as previously described (20). The level of resistance to NRTI-TP by the various RT mutants compared to that of corresponding wild-type RT was determined as IC₅₀ values of RT activity (Table 1 and Table 3). Resistance values (*n*-fold) were determined by dividing the IC₅₀ for the mutant by the IC₅₀ for the respective wild type. Steady-state kinetics could be assumed since the RT reaction was linear for more than 8 h (data not shown).

The NRTI-TP used were DOT-TP, DXG-TP, AZT-TP, carbocir (CBV)-TP (the active metabolite of abacavir), and TFV-DP. AZT-TP and TFV-DP were kindly provided by Cavid Tech. The other nucleotides were synthesized in our laboratory using a rapid method from the corresponding nucleoside analog (21). Purity (>92%) was confirmed by high-performance liquid chromatography and mass spectrometry.

prA/odT primer unblocking assay. The prA/odT assay, previously described (19, 20, 32), was used to further investigate the ATP-dependent primer unblocking for the RT mutants containing TAM and residue 69 insertion. This assay is limited to thymidine analogs, as prA₃₀₀ was used as the RNA template, and thereby only DOT-TP and AZT-TP were studied. In the reaction mixture, odT₁₂₋₁₈ (Sigma, St. Louis, MO) was added as primer together with BrdUTP (used as the only dNTP). Besides these changes, the same reaction buffer was used as for the DNA polymerase assay, together with the same procedure and assay time, but the NaOH step was omitted. The concentration of odT₁₂₋₁₈ was 0.22 ng/well (0.2 nM), since this odT amount was on the plateau of the *V*_{max} curve (95% relative *V*_{max}) when was studied the *K*_m of odT (data not shown). A lower ATP concentration (3.2 mM) than in the DNA polymerase assay was used, since previous studies (19, 20) had indicated that this amount was enough to display sufficient primer unblocking. The *K*_m (BrdUTP) and *K*_i (DOT-TP or AZT-TP) values were obtained by fitting the data to the Michaelis-Menten competitive inhibition equation using Grafit 5.0 (Erithacus Software, Horley Surrey, United Kingdom). The level of resistance by the RT mutant was expressed as an increase of the *K*_i/*K*_m value compared to the corresponding wild-type ratio. The concentration ranges of BrdUTP and DOT-TP or AZT-TP are described in Table 2. The RT amount used was 4 to 12 pM/well. The RT activity

TABLE 1. Resistance to NRTI-TP of RT mutants by the DNA polymerase assay^a

Mutations in respective wild-type background	NRTI-TP used ^b									
	DOT-TP		AZT-TP		DXG-TP		CBV-TP		TFV-DP	
	IC ₅₀ ± SE	Fold increase	IC ₅₀ ± SE	Fold increase	IC ₅₀ ± SE	Fold increase	IC ₅₀ ± SE	Fold increase	IC ₅₀ ± SE	Fold increase
Assay with ATP										
Wild-type HXB2(a)	0.38 ± 0.04	1.0	0.46 ± 0.05	1.0	0.15 ± 0.03	1.0	1.5 ± 0.6	1.0	3.3 ± 0.2	1.0
184V	0.5 ± 0.05	1.3	0.52 ± 0.1	1.1	0.2 ± 0.04	1.3	8.4 ± 2 ^c	5.6	2.3 ± 0.2 ^c	0.7
41L/67N/70R/210W/215Y	0.5 ± 0.2	1.3	2.3 ± 0.7 ^c	5.0	0.12 ± 0.03	0.8	1.5 ± 0.1	1.0	13 ± 2 ^c	3.7
41L/67N/70R/184V/210W/215Y	0.7 ± 0.03 ^c	1.8	0.9 ± 0.3	2.0	0.19 ± 0.05	1.3	4.5 ± 1 ^c	3.0	5.9 ± 0.7 ^c	1.8
41L/69S-SG/210W/215Y	1.6 ± 0.2 ^c	4.2	4.4 ± 1.2 ^c	9.6	0.34 ± 0.04 ^c	2.4	5.1 ± 0.05 ^c	3.4	24 ± 5 ^c	7.3
41L/69S-SG/184V/210W/215Y	1.4 ± 0.6 ^c	3.7	1.2 ± 0.8	2.6	0.26 ± 0.02 ^c	1.6	6.4 ± 0.9 ^c	4.3	ND	ND
75I/77L/116Y/151M	6.1 ± 2 ^c	16	9 ± 1 ^c	20	1.2 ± 0.5 ^c	10	11 ± 2 ^c	7.3	11 ± 2 ^c	3.3
75I/77L/116Y/151M/184V	15 ± 6 ^c	39	22 ± 3 ^c	48	2.4 ± 0.05 ^c	18	18 ± 7 ^c	12	4.6 ± 0.9	1.4
Wild-type HXB2(b)										
41L/67N/70R/215Y/219Q ^d	0.38 ± 0.02	1.0	0.48 ± 0.1	1.0	ND	ND	ND	ND	3.2 ± 0.3	1.0
	0.84 ± 0.1 ^c	2.2	2.5 ± 0.2 ^c	5.2	ND	ND	ND	ND	10.5 ± 2 ^c	3.3
Wild-type LAI										
65R ^d	0.19 ± 0.01	1.0	0.3 ± 0.06	1.0	0.2 ± 0.01	1.0	0.9 ± 0.5	1.0	1.3 ± 0.2	1.0
	0.75 ± 0.2 ^c	3.9	0.61 ± 0.1 ^c	2.0	1.3 ± 0 ^c	6.5	5.5 ± 2 ^c	6.1	21 ± 4 ^c	16
Wild-type pNL4-3										
115F ^d	0.3 ± 0.03	1.0	0.42 ± 0.1	1.0	ND	ND	1.8 ± 0.1	1.0	4 ± 0.5	1.0
	0.2 ± 0.05 ^c	0.6	0.55 ± 0.3	1.3	ND	ND	3.6 ± 0 ^c	2.0	3.7 ± 0.7	0.9
Assay without ATP										
Wild-type HXB2(a)	0.11 ± 0.05	1.0	0.18 ± 0.02	1.0	0.12 ± 0.02	1.0	1.1 ± 0.4	1.0	1.6 ± 0.3	1.0
184V	0.18 ± 0.06	1.6	0.25 ± 0.1	1.4	0.2 ± 0.05	1.7	5.5 ± 0.6 ^c	5.0	2.2 ± 0.2 ^c	1.4
41L/67N/70R/210W/215Y	0.12 ± 0.04	1.0	0.14 ± 0.03	0.8	0.11 ± 0.02	0.9	0.9 ± 0.02	0.8	1.4 ± 0.3	0.9
41L/67N/70R/184V/210W/215Y	0.16 ± 0.03	1.5	0.34 ± 0.1 ^c	1.9	0.14 ± 0.01	1.2	2.7 ± 0.1 ^c	2.5	1.4 ± 0.1	0.9
41L/69S-SG/210W/215Y	0.11 ± 0.01	1.0	0.16 ± 0.02	0.9	0.15 ± 0.01	1.3	0.9 ± 0.3	0.8	3.7 ± 0.4 ^c	2.3
41L/69S-SG/184V/210W/215Y	0.22 ± 0.03 ^c	2.0	0.21 ± 0.04	1.2	0.14 ± 0.04	1.2	3.4 ± 0.04 ^c	3.1	ND	ND
75I/77L/116Y/151M	2.1 ± 1 ^c	19	5.1 ± 0.6 ^c	28	2.5 ± 1 ^c	21	6.6 ± 3 ^c	6.0	8 ± 1 ^c	5.0
75I/77L/116Y/151M/184V	7.1 ± 2 ^c	65	10.6 ± 2 ^c	59	7.2 ± 2 ^c	60	54 ± 5 ^c	49	2.9 ± 0.3 ^c	1.8
Wild-type HXB2(b)										
41L/67N/70R/215Y/219Q ^d	0.12 ± 0.1	1.0	0.20 ± 0.04	1.0	ND	ND	ND	ND	ND	ND
	0.13 ± 0.02	1.1	0.19 ± 0.03	1.0	ND	ND	ND	ND	ND	ND
Wild-type LAI										
65R ^d	0.11 ± 0.02	1.0	0.08 ± 0.01	1.0	0.18 ± 0.01	1.0	0.68 ± 0.2	1.0	0.55 ± 0.1	1.0
	0.46 ± 0.2 ^c	4.2	0.35 ± 0.1 ^c	4.3	0.62 ± 0 ^c	3.4	5 ± 0.6 ^c	7.4	12 ± 0.4 ^c	22
Wild-type pNL4-3										
115F ^d	0.10 ± 0.01	1.0	0.13 ± 0.01	1.0	ND	ND	1.2 ± 0.2	1.0	1.2 ± 0.05	1.0
	0.08 ± 0 ^c	0.8	0.13 ± 0.02	1.0	ND	ND	2.6 ± 0.4 ^c	2.2	1.1 ± 0.1	0.9

^a Resistance of RT mutants to NRTI-TP was measured with the DNA polymerase assay of RT, with and without ATP (5 mM).^b The IC₅₀ values are expressed as μM of NRTI-TP. The IC₅₀ are averages from at least two separate experiments conducted in duplicate. The IC₅₀ values were determined using seven different concentrations of NRTI-TP adjusted optimally for each mutant's expected IC₅₀ value. Standard errors (± SE) are indicated. ND, not determined. Fold increase was calculated by dividing the mutant RT IC₅₀ values by the respective wild-type IC₅₀ values.^c Differs statistically from matched wild-type IC₅₀ (*P* < 0.05).^d Mutations 41L/67N/70R/215Y/219Q, 65R, and 115F in HXB2(b), LAI, and pNL4-3 background, respectively.

TABLE 2. Resistance of RT mutants to DOT-TP and AZT-TP measured with the prA/odT assay^a

Mutations in respective wild-type background	NRTI-TP ^b							
	DOT-TP				AZT-TP			
	$K_i \pm SE$	$K_m \pm SE$	K_i/K_m	Fold increase	$K_i \pm SE$	$K_m \pm SE$	K_i/K_m	Fold increase
Wild-type HXB2(a)	0.0028 \pm 0.001	0.32 \pm 0.04	0.0088	1.0	0.0054 \pm 0.0008	0.31 \pm 0.06	0.017	1.0
41L/67N/70R/210W/215Y	0.013 \pm 0.001	0.51 \pm 0.1	0.025	2.8	0.13 \pm 0.04	0.43 \pm 0.04	0.3	17.6
41L/69S-SG/210W/215Y	0.059 \pm 0.007	0.5 \pm 0.03	0.12	13.6	0.33 \pm 0.008	0.53 \pm 0.04	0.62	36.5
Wild-type HXB2(b)	0.0018 \pm 0.0005	0.24 \pm 0.03	0.0075	1.0	0.004 \pm 0.0004	0.29 \pm 0.004	0.014	1.0
41L/67N/70R/215Y/219Q ^c	0.011 \pm 0.002	0.42 \pm 0.04	0.026	3.5	0.095 \pm 0.02	0.49 \pm 0.03	0.19	13.6

^a Resistance of RT mutants to DOT-TP and AZT-TP relative to that of the wild type, measured with the prA/odT assay, with 3.2 mM ATP.

^b The K_i values are expressed in μ M of DOT-TP or AZT-TP and K_m values as μ M of BrdUTP. All values are the averages calculated from at least two separate experiments conducted in duplicate. Standard errors (SE) are indicated. Fold increase values were calculated as K_i/K_m values relative to those of the wild-type corresponding ratio. The K_m values were determined by using six different concentrations of BrdUTP, from 0.074 μ M to 16 μ M, in approximately threefold increments. The K_i values were determined for DOT-TP/AZT-TP by using four different concentrations adjusted optimally for each mutant's expected K_i value.

^c In the HXB2(b) background, from a different vector and purification method.

was linear during the assay time within the substrate range used, and thus, steady-state kinetics were assumed.

Statistical analyses. Average IC_{50} values in Table 1 and Table 3 were calculated using data that contained between two and four separate experiments, conducted in duplicate. The IC_{50} values of mutants were compared with the matched wild-type IC_{50} values, using at least four observations (pooled data). Outcomes were considered to differ statistically from matched wild-type IC_{50} values if P values of <0.05 were calculated based on the two-tailed Student's t test with equal variances.

RESULTS

A panel of 10 site-directed RT mutants was studied with the DNA polymerase assay. The novel inhibitors DOT-TP and DXG-TP (as T and G analogs) were compared to the antiviral NRTI-TP of currently approved drugs such as AZT-TP, CBV-TP, and TFV-DP (as T, G, and A analogs). The results are described below and are summarized in Table 1. The substrate discrimination effect was measured in assays without ATP and the primer unblocking effect by adding ATP in the RT assay. Hence, it is the combined resistance effect of substrate discrimination and primer unblocking, i.e., measured in the presence of ATP, that balance out to the final data related to the resistance level observed.

Mutations involved in ATP-dependent primer unblocking. The levels of resistance of the 41/67/70/210/215 mutant in the HXB2(a) background and the 41/67/70/215/219 mutant in the HXB2(b) background were determined. As expected for these TAM mutants, increased resistance to NRTI was found only in the presence of ATP (Table 1). Thus, in the absence of ATP, these mutants behaved like the wild type, i.e., they were involved only in primer unblocking and not in substrate discrimination (Table 1). When ATP (5 mM) was added to the DNA polymerase assay, the order of fold increase for the two TAM mutants was AZT-TP (5.0- and 5.2-fold, respectively) $>$ TFV-DP (3.7- and 3.3-fold, respectively) $>$ DOT-TP (1.3- and 2.2-fold, respectively) $>$ CBV-TP (1.0-fold) $>$ DXG-TP (0.8-fold) (Table 1). In addition, the 41/67/70/210/215 mutant with d4T-TP resulted in a 4.1-fold level of resistance (data not shown). The same order of increase of the NRTI-TP was found with the 41/69S-SG/210/215 mutant enzyme. Addition of the residue 69 insertion gave a more pronounced primer unblocking for all analogs, which is in agreement with previous studies (4, 19, 20, 23).

It is known that incorporation of a dNTP to the next nucleotide position on the template can prevent primer unblocking

TABLE 3. The DNA polymerase assay with 5 mM ATP and with modification of dNTP concentration or template sequence

Mutations in wild type background	Assay modifications of dNTP concn or template sequence	NRTI-TP ^a					
		DOT-TP		TFV-DP		AZT-TP	
		$IC_{50} \pm SE$	Fold increase	$IC_{50} \pm SE$	Fold increase	$IC_{50} \pm SE$	Fold increase
Wild-type HXB2(a)	1 μ M dNTP	0.38 \pm 0.04	1.0	3.3 \pm 0.2	1.0	0.46 \pm 0.05	1.0
Wild-type HXB2(a)	20 μ M dNTP	2.0 \pm 0.1	1.0	19 \pm 0.5	1.0	3.3 \pm 0.7	1.0
41L/67N/70R/210W/215Y	1 μ M dNTP	0.5 \pm 0.2	1.3	13 \pm 2 ^b	3.7	2.3 \pm 0.7 ^b	5.0
41L/67N/70R/210W/215Y	20 μ M dNTP	2.4 \pm 0.3	1.2	49 \pm 7 ^b	2.6	16 \pm 3 ^b	4.8
Wild-type HXB2(a)	5'-A ₁₂ -(GTCA) ₅ -3'	0.38 \pm 0.04	1.0	3.3 \pm 0.2	1.0	0.46 \pm 0.05	1.0
Wild-type HXB2(a)	5'-A ₁₂ -(GACT) ₅ -3'	0.31 \pm 0.01	1.0	2.8 \pm 0.3	1.0	ND	ND
41L/67N/70R/210W/215Y	5'-A ₁₂ -(GTCA) ₅ -3'	0.5 \pm 0.2	1.3	13 \pm 2 ^b	3.7	2.3 \pm 0.7 ^b	5.0
41L/67N/70R/210W/215Y	5'-A ₁₂ -(GACT) ₅ -3'	0.4 \pm 0.03 ^b	1.3	8.6 \pm 1 ^b	3.1	ND	ND
41L/69S-SG/210W/215Y	5'-A ₁₂ -(GTCA) ₅ -3'	1.6 \pm 0.2 ^b	4.2	24 \pm 5 ^b	7.3	4.4 \pm 1.2 ^b	9.6
41L/69S-SG/210W/215Y	5'-A ₁₂ -(GACT) ₅ -3'	0.78 \pm 0.1 ^b	2.5	17 \pm 1 ^b	6.0	ND	ND

^a Mean IC_{50} values \pm standard errors ($\pm SE$) are expressed as μ M from at least two separate experiments conducted in duplicate. ND, not determined. Fold increase was calculated by dividing the mutant RT IC_{50} values by respective wild-type IC_{50} values and assay modification.

^b Differs statistically from matched wild-type IC_{50} ($P < 0.05$) values.

by a so-called dead-end-complex (DEC) (26). Therefore, we studied the 41/67/70/210/215 mutant by using higher dNTP concentrations (20 μ M) and found a similar increase toward that of the wild-type level for DOT-TP and AZT-TP but a lower resistance (2.6-fold) for TFV-DP (Table 3).

It has been reported that the upstream template sequence can have an effect on ATP-dependent primer unblocking (22). Therefore, the effects on primer unblocking of DOT-MP and TFV were determined with the RT mutants 41/67/70/210/215 and 41/69S-SG/210/215 in an assay with a different template sequence, i.e., a repeat sequence of 5'-(GACT)₅3' instead of the 5'-(GTCA)₅3' sequence described in Materials and Methods. A similar or a slightly lower range of *n*-fold increased values was found for each of the TAM mutants DOT-TP (1.3- and 2.5-fold, respectively) and TFV-DP (3.1 and 6.0-fold, respectively) (Table 3).

A further investigation of DOT-TP and AZT-TP resistance was performed with the prA/odT assay. As shown in Table 2, the levels of resistance, measured as the mutant K_i/K_m ratio over that of the wild type, were overall higher for both nucleotides. However, DOT-TP demonstrated a smaller *n*-fold increase for the two TAM mutant enzymes (2.8- and 3.5-fold, respectively) in comparison with that of AZT-TP (17.6- and 13.6-fold, respectively), and the same trend applied for the residue 69 insertion mutant (13.6- compared to 36.5-fold, respectively).

Mutations involved in non-ATP-dependent discrimination. The DNA polymerase assay with and without ATP was used to study the resistance mechanism involved in the reduced ability to incorporate NRTI relative to natural substrate (Table 1). A large increase of non-ATP-dependent DOT-TP resistance was found with the Q151M mutants, from 16- to 65-fold, depending on the inclusion of the M184V mutation and on added ATP (Table 1). RTs carrying the K65R mutation appeared to be associated with some decreased discrimination to DOT-TP with and without ATP (3.9- and 4.2-fold, respectively). However, this decrease was less than that for CBV-TP (6.1- and 7.4-fold, respectively) and not of the same magnitude as that for TFV-DP (16- and 22-fold, respectively). The RT carrying the M184V mutation alone did not exhibit any apparent resistance for the NRTI-TP studied, except for CBV-TP (5-fold). The abacavir-associated mutation Y115F showed no resistance against DOT-TP, and only a twofold binding discrimination against CBV-TP was noted (Table 1).

Mutations involved in the suppression of resistance. Addition of the M184V mutation to the 41/67/70/210/215 mutant suppressed resistance to AZT-TP from 5.0- to 2.0-fold and resistance to TFV-DP from 3.7- to 1.8-fold (Table 1). Furthermore, a comparison of results from assays performed in the presence and absence of ATP indicated slightly lower resistance levels with RTs bearing only the M184V mutation for all NRTI (except for CBV-TP) (Table 1). This reduced effect of M184V in mutants without TAM seemed more evident with the 75/77/116/151M/184V mutant in the presence of ATP. The most prominent reductions were observed for DXG-TP and CBV-TP. However, the 75/77/116/151M mutant by itself without M184V was also found to be involved in decreased resistance to almost the same degree. Therefore, a comparison of assay results in the presence and absence of ATP suggests that Q151M and its family of mutations may contribute toward a

suppressed resistance profile in a manner similar to that of M184V. Furthermore, the same ATP-dependent suppression effect was obtained with the K65R mutation for most NRTI-TP.

DISCUSSION

DOT was previously found to have a more potent activity than AZT and TFV against viruses with TAM in studies using primary human lymphocytes (9, 30). In the present study, we confirmed that DOT-TP had greater activity against RTs with TAM than against AZT-TP and TFV-DP, using the DNA polymerase assay (Table 1). To further explore this difference, extra dNTPs (20 μ M) were added in the assay. It is known that high AZT resistance in cell-based systems expressing TAM in HIV-1 RT can be attributed to AZT's bulky azido group, preventing DEC formation (26). The formation of the DEC (in the presence of high dNTP concentration) inhibits ATP removal of incorporated NRTI-MP. This has previously been demonstrated for d4T-MP and TFV (but not for AZT-MP) with RTs bearing TAM (26, 27). In the DNA polymerase assay, the lower sensitivity to dNTP inhibition for the 41/67/70/210/215 mutant on primers chain terminated with AZT-MP was assessed. A 30% higher sensitivity for dNTP inhibition with chain-terminated TFV was noted (Table 3), while the TAM mutant was generally less resistant to DOT-TP, irrespective of the dNTP concentration used. Since the intracellular dNTP concentration is estimated to be 0.3 to 5 μ M in resting peripheral blood mononuclear cells and 3 to 26 μ M in activated cells (13, 36), the 1 μ M of the dNTP used in the DNA polymerase assay (Table 1) more closely represented the human situation, whereas the 20 μ M used (Table 3) more closely represented the situation in cell culture assays. It should be noted that RTs bearing the residue 69 insertion mutations have a decreased sensitivity to dNTP inhibition of the primer unblocking activity, which could explain why these mutants give higher and broader NRTI resistance in both the cell culture and the RT assay (23).

It is known that the template sequence has an effect on the ATP-dependent primer unblocking (22). In particular, large variations have been reported for primers terminated with a ddA-MP, ddC-MP, or ddG-MP analog, while T analogs such as AZT-MP and ddT-MP were found to be markedly less influenced by the template sequence (22). Therefore, our study was limited to only two repeats (GACT and GTCA). Comparisons of DOT-TP and TFV-DP using RTs bearing TAM or TAM with residue 69 insertion suggest that primer-incorporated DOT-MP maintains a favorable reduced ability for primer unblocking (Table 3).

Kinetic parameters from our previous studies of RT with TAM and residue 69 insertions, using a prA/odT assay, had revealed an approximately twofold higher AZT-TP resistance than the data for DNA polymerase as presented above (20). In the present study, using the prA/odT assay, a higher ATP-dependent resistance for AZT-TP and DOT-TP was found (Table 1 and Table 2). A larger primer unblocking capacity between the mutant and the wild-type RTs in the prA/odT assay than in the DNA polymerase assay may be explained by the long homopolymeric prA₃₀₀ template strands, which allow more multiple incorporations than the shorter heteropolymeric DNA₃₂ template. It is noteworthy that the present prA/

odT assay study generated an almost twofold higher AZT-TP resistance than the previous AZT-TP data described, using a similar prA/odT assay with related mutants (20). This may have resulted from using a new type of prA plate (High Sensitivity) and/or a different assay buffer condition. Irrespective of the DNA or prA/odT assay used, the primer unblocking rate of incorporated DOT-MP was, overall, fourfold lower than the removal of AZT-MP in RTs bearing TAM.

High levels of resistance to DOT-TP and DXG-TP by RT bearing the Q151M mutation were found through a non-ATP-dependent discrimination mechanism (Table 1). This is in agreement with the high levels of DOT resistance observed for viral drug susceptibility cell-based assays using virus containing the Q151M and similar family mutations (30). This suggested that the methionine residue 151 could make direct contact with the incoming DOT's dioxolane sugar ring. In contrast, the multidrug-resistant Q151M family mutations demonstrated very low resistance against TFV-DP compared to that of other NRTI (Table 1), in agreement with virus drug susceptibility assay data (31).

A fourfold range of DOT-TP resistance was found with the K65R mutant, both with and without ATP, which was lower than that for CBV-TP and TFV-DP (Table 1). However, the AZT-TP data also showed a somewhat elevated resistance (2-fold with ATP and 4.3-fold without ATP). Since the K65R mutant has previously been reported to be susceptible to AZT (onefold resistance or less) (28, 37), the data are somewhat unclear on how important K65R is for discriminating DOT-TP.

It is well recognized that mutations M184V and K65R diminished the ability of HIV-1 RT to perform the primer unblocking of incorporated NRTI-MP such as AZT-MP (6, 14, 37). It is thereby established that M184V and K65R mutants have less primer unblocking ability than wild-type RT (6, 14, 37). A comparison of the results in the presence and absence of ATP demonstrated that primer unblocking was decreased in the presence of K65R or M184V, which resulted in an overall lower NRTI-TP resistance in the presence of ATP (Table 1). Furthermore, we demonstrated the novel finding that Q151M and its family of mutations (without M184V) also had this ability (Table 1). It is notable that mutations involved in the suppression of primer unblocking clearly demonstrate that the final resistance level is dependent upon both resistance mechanisms (substrate discrimination and primer unblocking) working in concert. Furthermore, the ability of the 41/67/70/210/215 mutant to enable primer unblocking of incorporated AZT-MP and TFV was reduced with the addition of the M184V mutation (Table 1 [with ATP]). Surprisingly, the presence of mutation M184V in combination with Q151M and family mutations significantly increased the binding discrimination for DOT-TP, AZT-TP, and DXG-TP (also CBV-TP, but not TFV-DP), in comparison to that of the Q151M mutant without M184V (Table 1). However, an increase (from 13- to 21-fold) in AZT resistance for the same clones was noted when studied in a viral drug susceptibility assay (unpublished data).

It is hypothesized that the abacavir-associated mutation Y115F (tyrosine 115 phenylalanine) could be involved in DOT resistance and give rise to a different resistance profile. It has previously been predicted, using modeling simulations of the three-dimensional structure of HIV-1 RT, that the amino backbone of the tyrosine residue 115 could stabilize the 3'-oxygen

in the D-like dioxolane ring (9). This could well be the case, but the mutation switch occurs in the side chain and not in the backbone. It is known that the side chain of tyrosine 115 interacts with the 2' group in the deoxynucleotide sugar moiety and that a switch to the smaller phenylalanine side chain can induce misincorporation of ribonucleotides (2). The phenylalanine 115 residue apparently discriminates CBV-TP slightly but not DOT-TP. We are currently selecting for resistance mutations toward DOT in HIV-1-infected primary human lymphocytes. To date, after 40 passages in the presence of DOT, no mutation has been selected.

In summary, the facile nonradioactive DNA polymerase assay proved useful for studies of the mechanism of HIV-1 resistance to DOT-TP and provided comparative data for the level of DOT-TP resistance to other NRTI-TP. The biochemical mechanism of HIV-1 resistance to NRTI-TP occurs in two different ways depending on the amino acid substitutions present in the RT. The data suggest that the accumulation of TAM involving primer unblocking can result in a low level of resistance to DOT-TP and that this level was found to be lower for AZT-TP and TFV-DP. The mechanism involving non-ATP-dependent discrimination demonstrated that RT bearing the Q151M cluster was highly resistant to DOT-TP, whereas discrimination was not found with RT bearing only the mutation M184V or Y115F. DOT-TP was more potent against HIV-1 RT containing K65R than TFV-DP and CBV-TP. Therefore, DOT-TP showed a resistance profile similarly favorable to that of DXG-TP. In view of these data, DOT merits further development as a potential TK1-dependent anti-HIV-1 agent.

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